Madroño, Vol. 55, No. 4, pp. 269–279, 2008

AN *ERIOPHYLLUM LANATUM* (ASTERACEAE) HYBRID ZONE IN OREGON

John S. Mooring
Biology Department, Santa Clara University, Santa Clara, CA 95053
jmooring@scu.edu

Abstract

Eriophyllum lanatum vars. *achilleoides* and *leucophyllum* intergrade in southwestern Oregon. Some populations cannot be unequivocally assigned to either variety. Chromosome counts showed polyploid populations predominating where the varieties meet in southwestern Oregon. Pollen fertility, estimated by cotton blue-lactophenol staining, was the main criterion used to assess barriers to interbreeding. Artificial hybridizations between diploids revealed barriers to interbreeding between vars. *achilleoides* and *leucophyllum*, and between each of them and a morphologically intermediate population. The barriers to interbreeding are substantially less developed than those present between most of the other eight varieties of the *E. lanatum* complex. Supernumerary chromosomes are postulated to be adaptive in *Eriophyllum* hybrid zones.

Key Words: Artificial hybridizations, Asteraceae, barriers to interbreeding, *Eriophyllum*, hybrid zone, pollen fertility, species complex, supernumerary chromosomes.

I dedicate this contribution to the memory of Harlan Lewis, mentor and friend.

The *Eriophyllum lanatum* (Pursh) J. Forbes species complex extends from California to British Columbia and eastward to Utah, Montana, and Wyoming. The base chromosome number is $x = 8$. One to four supernumerary chromosomes may be present. Diploid, tetraploid, hexaploid, and octoploid populations occur from coastal to subalpine communities.

Artificial hybridizations showed significant differences in pollen fertility in the progeny of some intervarietal crosses, indicating barriers to interbreeding. The barriers were higher in diploids than in tetraploids. The "varieties" are geographic subspecies or semispecies. Many of the polyploid populations occur where the ranges of taxa overlap or nearly overlap (Mooring 2001). Assigning individuals or populations to taxa is often difficult in such areas. The morphological variability and the high frequency of polyploid populations in these areas strongly suggest that they are hybrid zones.

Particularly good examples of taxonomic uncertainties occur in the Klamath Region of northwestern California and southwestern Oregon where *E. lanatum* vars. *achilleoides* (DC) Jeps., *grandiflorum* (A. Gray) Jeps., *lanceolatum* (J. T. Howell) Jeps., and *leucophyllum* (DC) W. R. Carter occur. Constance (1937, p. 89) wrote that "it seems quite impossible to assign many specimens definitely to any one subspecific category." In particular, vars. *achilleoides* and *leucophyllum* intergrade smoothly (Constance 1937; Kenton Chambers, Dept. Of Botany and Plant Pathology, Oregon State University, oral communication).

The var. *achilleoides*-var. *leucophyllum* transition region in southwestern Oregon offered an opportunity to plot the distribution of diploid and polyploid populations and to examine the extent of barriers to interbreeding between the two taxa. Mooring (2001) had reported three diploid and two tetraploid populations in Curry, Douglas, Jackson, and Josephine Counties. The Douglas County population, a tetraploid, was clearly var. *leucophyllum*, three other populations were var. *achilleoides*. The fifth population a diploid, was somewhat intermediate, but assigned to var. *achilleoides*. The only artificial hybridization between vars. *achilleoides* and *leucophyllum* did not use populations from southwestern Oregon (Mooring 2001, Appendix 1). A more intensive biosystematic study of both varieties in the Pacific Northwest could assess barriers to interbreeding and might suggest the extent of the hybrid zone. Here, I report the results of cytogeographic and artificial hybridization studies in *E. lanatum* vars. *achilleoides* and *leucophyllum* in Oregon and Washington, and discuss the results in the context of a previous study (Mooring 2001).

Materials and Method

Plants

Fruits or plants were collected from the populations listed in Appendices 1 and 2.

Growth Conditions

Black and thick fruits were germinated in vermiculite or in vermiculite-soil mixtures. Seedlings were potted in commercial potting mixtures, "UC Soil Mix" or Orchard Supply Hardware soil mix with fertilizer (12% N, 12% P, 6% K) added. The latter mix gave better results. Seedlings were grown in an unheated greenhouse at Santa Clara University. Most seedlings were transplanted to a local garden within 5–6 mo because they outgrew the pots and were damaged by white flies. Most garden plants were allowed to grow for at least two years (until they died of natural causes or had to be removed because space was needed for another crop).

Meiotic Analyses

Young capitula of wild, garden, or greenhouse plants were fixed in 1:3 acetic ethanol or in 1:3:6 acetic-chloroform-ethanol. Quickly placing collections in an ice-filled cooler usually improved fixation (Anderson 1966). Microsporocytes in acetocarmine or aceto-orcein squashes were examined by light or phase contrast microscopy at diakinesis or first metaphase. Aceto-orcein usually gave better preparations.

Artificial Hybridizations

Because some northwestern plants did not flower synchronously or at all, only 14 hybrid combinations were possible. Bagging experiments and decades of growing plants of this species complex show the plants to be highly self-incompatible. Pollination was done by rubbing together capitula of isolated greenhouse plants over 3–8 d.

Pollen Fertility Estimates

Pollen was sprinkled on a slide, a drop of cotton blue-lactophenol was added and the drop was swirled around before the coverslip was applied. The grains were stained overnight before being examined at 100× to 400× magnification. Plump, dark blue grains were scored as viable. Over 98% of the estimates rest on 300+ grains per sample, with each plant being sampled twice, on different days. No sample had fewer than 150 grains.

Statistical Analyses

Statistical significance of differences in percentage pollen fertility among progenies was assessed by a one-tailed t-test using a Sigmastat program.

RESULTS

Cytogeography

This study added chromosome counts for 16 populations from Oregon, 1 from Washington, and 1 from California (Appendix 1) to a previous report (Mooring 2001). All but three populations were var. *achilleoides* or var. *leucophyllum* or intermediates between these taxa. The counts included two diploid and two tetraploid populations of var. *achilleoides*, three diploid, three tetraploid, and one hexaploid population of var. *leucophyllum*, and one diploid, two tetraploid, and one hexaploid population of intermediates (Appendix 1). The exact number of chromosomes in the two hexaploid-level populations was unclear; determinations of 23–24 II and 23–25 II were entered for, respectively, Populations *334* and *346*. The count for Population *346* is a first report of a hexaploid in var. *leucophyllum*. Appendix 1 also lists a count of 8 II in var. *grandiflorum*, a first report for the occurrence of that variety in Oregon, and counts of 8 II for two populations of var. *lanceolatum*.

The diploid and polyploid populations found in the present study were not randomly distributed. In respect to var. *leucophyllum* populations, the diploids were in northernmost Oregon. Three of the four polyploid ones were in central and southwestern Oregon, interspersed with diploid and polyploid populations of var. *achilleoides* and populations intermediate between the two varieties. The intermediates are Populations *338*, diploid, *339* and *335*, tetraploid, and *334*, hexaploid (Appendix 1). Some individuals of the intermediates averaged taller than plants of nearby populations of var. *achilleoides* (*336*, *350*) and had ternately divided as well as pinnately divided leaves, similar to the description of *Eriophyllum ternatum* Greene (see Cronquist 1955, p. 196).

Supernumerary Chromosomes

Chromosomes in excess of the basic complement, here called "supernumerary" chromosomes, were present in diploid populations *338* and *355* and in tetraploid populations *335* and *337*. When two supernumeraries were present they remained unpaired or formed a bivalent. Three of the plants analyzed in Population *338* had one supernumerary chromosome; one plant had two (Appendix 1). These chromosomes were transmitted or increased in number in artificial hybridizations (Table 1, crosses 10 and 11).

Artificial Hybridizations

Of the 15 hybridizations reported in this study, aspects of those numbered 1, 2, 3, 4, 7, 9, 10, and 11 (Table 1) have been published in parts of a longterm investigation of the *E. lanatum* complex (Mooring 2001, 2007).

Germination. Germination varied more in diploid (3%–44%) than in tetraploid progenies (9%–22%) (Table 1). Mean germination, however, did not differ significantly between the diploid

(23% ± 14%) and the tetraploid progenies (18% ± 8%). Likewise, mean germination did not vary significantly in the progeny of diploid varietal combinations: 29% ± 9% for *achilleoides* × *achilleoides*, 16% ± 14% for *leucophyllum* × *leucophyllum*, 29% ± 22% for *achilleoides* × *leucophyllum*, and 15% ± 17% for *achilleoides-leucophyllum* intermediate × *achilleoides*. Percentage germination in the only *achilleoides-leucophyllum* intermediate × *leucophyllum* progeny was 14%.

Pollen fertility variability. Pollen fertility varied widely within most diploid progenies, up to 58 percentage points. Wide ranges were more frequent in Crosses 1, 2, 9, 10, and 11, which used parents from the Calistoga, California, or Dillard, Oregon, populations. Pollen fertility ranges in tetraploid progenies were much narrower, 3–20 percentage points (Table 1).

Mean pollen fertility for the progenies of the diploid varietal combinations was 80% ± 18% for *achilleoides* × *achilleoides*, 90% ± 11% for *leucophyllum* × *leucophyllum*, 74% ± 15% for *achilleoides* × *leucophyllum*, and 68% ± 13% for the *achilleoides-leucophyllum* intermediate × var. *achilleoides*. Pollen fertility was 81% ± 13% for the single *achilleoides-leucophyllum* intermediate × var. *leucophyllum* progeny. The mean of means for these varietal combinations was approximately the same as the means given above (Table 1).

Mean pollen fertility for the progenies in the three tetraploid combinations (86%–98%) was much higher than the diploid combinations (Table 1, Crosses 12, 13, and 14).

Mean pollen fertility in diploid progenies. Within the var. *achilleoides* × var. *achilleoides* progenies and within the var. *achilleoides* × var. *leucophyllum* progenies, mean pollen fertilities did not differ significantly (Table 2, Crosses 1–4 and 7–8). In the var. *leucophyllum* × var. *leucophyllum* progenies, mean pollen fertility was significantly lower ($P \leq 0.01$) in the Rowena × Shepperd's Falls progeny than in the Silver Falls × Rowena progeny (Tables 1, 2, Crosses 5 and 6). In the var. *achilleoides*-var. *leucophyllum* intermediate × var. *achilleoides* crosses, the mean pollen fertilities of the two progenies did not differ significantly (Table 1, Crosses 9 and 10), but the Dillard × Calistoga one (Cross 9) differed significantly ($P \leq 0.05$) from three of the four var. *achilleoides* × var. *achilleoides* progenies (Tables 1, 2, Crosses 1, 3, and 4).

In the single var. *achilleoides*-var. *leucophyllum* intermediate × var. *leucophyllum* cross, mean pollen fertility differed significantly ($P \leq 0.01$) from the var. *achilleoides*-var. *leucophyllum* intermediate × var. *achilleoides* progeny. The Population *338* parent in the two crosses came from different plants (Table 1, Crosses 9 and 11).

Parent-progeny pollen fertility differences. In the eight crosses among diploids where parental pollen fertility was known, mean pollen fertility in five progenies was lower by 11%–22%, and in three crosses it was higher by 1%–11%. In the three tetraploid crosses, pollen fertility equaled or was higher (19%, 25%) than that of the parents (Table 1).

Meiotic Pairing

Tetraploids were not examined. Diploids formed 8 II at diakinesis or metaphase in the progeny of six of the ten crosses where microsporocytes could be studied. In Crosses 6 and 7, 8 II was the norm, but some 7 II + 2 I configurations were seen, as well as one apparent bridge-fragment in Cross 7 (Table 1). One or two supernumerary chromosomes were present in the progeny of Crosses 9 and 10, where parent *S338-4* had one supernumerary chromosome, and Cross 11, where parent *S338-6* had two (Table 1).

DISCUSSION

Systematics

Eriophyllum lanatum var. *leucophyllum* (DC.) W. R. Carter extends from southwestern Oregon to the Columbia River, thence eastward to approximately Hood River, Oregon, north in the Cascade Mountains and coastal regions of Washington to southwestern British Columbia. To the east, var. *leucophyllum* intergrades with var. *lanatum*. Constance (1937) included var. *leucophyllum* in var. *lanatum*, considering it to be a western expression of var. *lanatum*. Mooring (2001), relying on morphological and ecological information, and using results from artificial hybridizations, believed that the western populations of var. *lanatum* (*sensu* Constance 1937) were best treated as var. *leucophyllum* (DC.) W. R. Carter. The results of artificial hybridizations using four populations indicated strong barriers to interbreeding between the var. *lanatum* and var. *leucophyllum* populations.

Variety *achilleoides* (DC.) Jepson ranges from central Oregon and extreme northwestern Nevada south to San Luis Obispo County in coastal California. In the North Coast Ranges of California, it intergrades with var. *arachnoideum* (Fisch. & Avé-Lall.) Jeps. to the west and with var. *grandiflorum* to the east. In the North Coast Ranges, 36 of 66 populations of vars. *achilleoides*, *arachnoideum*, and *grandiflorum* or intermediates between vars. *achilleoides* and *grandiflorum* or between vars. *achilleoides* and *arachnoideum* were polyploid, including 18 of the 19 intermediates (Mooring 2001).

Variety *achilleoides* consists of regional clusters of populations. Constance (1937, p. 87–88) ob-

TABLE 1. ARTIFICIAL HYBRIDIZATIONS IN THE *ERIOPHYLLUM LANATUM* COMPLEX. Crosses are numbered to facilitate discussion. Locations are state: (CA = California; OR = Oregon; WA = Washington) county, closest city. "Phase" refers to regional clusters of populations in var. *achilleoides*. Italicized numbers identify parents; the first number represents the population, the second a plant of that population. "S" refers to seed-grown plants, transplants from nature lack an "S." The seed parent is listed first. "R" = reciprocal cross. ND = no data or information available. "N" refers to the number of progeny furnishing pollen.

			Percentage stainable pollen					
			Parents	F1				
Cross – by location	Parents	Germination frequencies (%)	Mean	Range	Mean ± 1 SD	Difference from mean of parents, %	n	Meiosis, pairing at M1 or diakinesis
DIPLOID: VAR. *ACHILLEOIDES* × VAR. *ACHILLEOIDES*								
1. CA: Napa, Calistoga (Phase 1) × CA: Napa, Calistoga (Phase 1)	*118-12* × *118-18*	14/47 (30)	86, 94	50–98	80 ± 30	−11	12	8 II
2. CA: Napa, Calistoga (Phase 1) × CA: Lake, Kelseyville (Phase 1)	*118-11* × *120-13*	22/56 (39)	83, 82	40–98	73 ± 19	−12	12	ND
3. OR: Jackson, Prospect (Phase 2) × CA: Santa Clara, Los Gatos (Phase 1)	*S275-3* × *38*	19/104 (18)	98, ND	68–89	81 ± 8		5	8 II
4. CA: Lassen, Litchfield (Phase 2) × OR: Jackson, Prospect (Phase 2)	*S242-1* × *S275-3*	75/273 (27) Mean = 29% ± 9%	60, 98	69–95	85 ± 14 Mean = 80% ± 18%	+8	3	8 II
DIPLOID: VAR. *LEUCOPHYLLUM* × VAR. *LEUCOPHYLLUM*								
5a. OR: Marion, Silver Falls × OR: Wasco, Rowena	*341-3* × *342-2*	9/91 (10)	91, 97	84–100	96 ± 5	+1	7	8 II
5b. OR: Wasco, Rowena × OR: Marion, Silver Falls	*342-2* × *341-3*	3/51 (6)	97, 91	86–99	94 ± 7	0	3	ND
6. OR: Wasco, Rowena × OR: Multnomah, Shepperd's Falls	*S342-2* × *355-2*	36/113 (32) Mean = 16% ± 14%	99, ND	63–90	81 ± 12 Mean = 90% ± 11%		6	8 II and 7 II + 2 I
DIPLOID: VAR. *ACHILLEOIDES* × VAR. *LEUCOPHYLLUM*								
7. CA: Lake, Lower Lake (Phase 1) × OR: Multnomah, Bonneville	*S121-1* × *S167-2*	11/25 (44)	ND, ND	51–81	66 ± 11		5	8 II, a few 7 II + 2 I and bridge-fragment
8. OR: Curry, Gold Beach (Phase 2) × OR: Marion, Silver Falls	*347-5* × *341-1* & R	19/149 (13) Mean = 29% ± 22%	96, 92	50–94	78 ± 16 Mean = 74% ± 15%	−17	10	8 II
DIPLOID: *ACHILLEOIDES-LEUCOPHYLLUM* INTERMEDIATE × VAR. *ACHILLEOIDES*								
9. OR: Douglas, Dillard × CA: Napa, Calistoga (Phase 1)	*S338-4* × *S118-5-2*	21/79 (27)	80, 96	50–89	69 ± 11	−22	20	8 II, 8 II + 1 I, 8 II + 2 I

TABLE 1. CONTINUED

			Percentage stainable pollen					
			Parents	F1				
Cross – by location	Parents	Germination frequencies (%)	Mean	Range	Mean ± 1 SD	Difference from mean of parents, %	n	Meiosis, pairing at M1 or diakinesis
10. OR: Douglas, Dillard × OR: Josephine, Galice (Phase 2)	*S338-4* × *S350-13* & R	15/595 (3) Mean = 15% ± 17%	80, 78	51–94	64 ± 20 Mean = 68% ± 13%	−19	4	8 II, 8 II + 1 I
DIPLOID: *ACHILLEOIDES-LEUCOPHYLLUM* INTERMEDIATE × VAR. *LEUCOPHYLLUM*								
11. OR: Douglas, Dillard × OR: Wasco, Rowena	*S338-6* × *S342-6*	22/158 (14)	61, 85	43–94	81 ± 13	+11	16	8 II, 8 II + 1 I, 8 II + 2 I
TETRAPLOID: VAR. *ACHILLEOIDES* × VAR. *ACHILLEOIDES*								
12. CA: Colusa, Wilbur Springs (Phase 1) × OR: Curry, Rogue River (Phase 2)	*S149-1* × *S348-5* & R	38/171 (22)	98, 99	94–100	98 ± 2	0	6	ND
13. OR: Jackson, Central Point (Phase 2) × OR: Curry, Rogue River (Phase 2)	*S335-7* × *S348-5* & R	87/405 (22)	62, 99	94–97	96 ± 2	+19	2	ND
TETRAPLOID: VAR. *ACHILLEOIDES-LEUCOPHYLLUM* INTERMEDIATE × VAR. *LEUCOPHYLLUM*								
14. OR: Jackson, Central Point × WA: Wahkiakum, Cathlamet	*S335-7* × *S344-1* & R	12/141 (9) Tetraploid mean = 18% ± 8%	62, 75	77–97	86 ± 7 Tetraploid mean = 93% ± 6%	+25	5	ND

TABLE 2. STATISTICALLY SIGNIFICANT DIFFERENCES IN POLLEN FERTILITY BETWEEN CROSSES. Crosses are numbered as in Table 1.

Cross comparison	P value
5. Rowena × Silver Falls **vs.** 6. Rowena × Shepperd's Falls	<0.01
3. Prospect × Los Gatos **vs.** 7. Lower Lake × Bonneville	<0.05
9. Dillard × Calistoga **vs.** 1. Calistoga × Calistoga	<0.05
9. Dillard × Calistoga **vs.** 3. Prospect × Los Gatos	<0.05
9. Dillard × Calistoga **vs.** 4. Litchfield × Prospect	<0.05
9. Dillard × Calistoga **vs.** 11. Dillard × Rowena	<0.01

served that two major groups of populations were present, the "typical race" of the North Coast Ranges and a "somewhat different population" of the Klamath region of California and Oregon. My conclusions differ; three major and widespread intergrading regional phases can be distinguished in var. *achilleoides*, as well as one local phase. Phase 1 occurs from San Luis Obispo County to approximately Tehama County in California. Phase 2 occurs in northwestern California and southwestern Oregon (the Klamath area). Phase 3 occupies more arid regions than Phase 2, with which it smoothly intergrades, occurring in northeastern California, the northwestern corner of Nevada, and southeastern Oregon as far north as Harney County. Generally, Phase 3 populations consist of smaller plants than those of Phase 2 (some populations appear to have been introgressed by var. *integrifolium* (Hook.) Smiley.) The local phase, including some populations treated as *Eriophyllum Cusickii* Eastw. by Eastwood and *E. ternatum* by Greene (Constance 1937, p. 88) centers in the Ashland to Medford area of southwestern Oregon. Cronquist (1955, p. 196) noted that taller plants, often with ternately divided leaves, replaced individuals with pinnately divided leaves, and commented they "are possibly best considered a distinct variety." Morphologically, these populations appear to provide a continuum between vars. *achilleoides* and *leucophyllum*, being intermediate in respect to characters that can be used to distinguish modal populations of the two varieties (Table 3).

A massive root system, abundance of root buds, and the clumped nature of the plants characterized the natural populations of var. *leucophyllum* that I examined. Some of these populations appeared to consist of one or more clones. What appeared to be separate plants were connected by tough roots (not rhizomes), e.g., *337*, *340*. The characters noted in Table 3 largely persisted in cultivation. The massive root system was especially noticeable. The root system of a single plant, and the shoots that arose from root buds, usually filled a 5-inch pot so completely that it was difficult to remove the plant. In garden culture, individuals had a diameter of up to 1.5 m, and spread by new shoots arising from root buds. Some garden plants produced 15–30 flowering stems, others had not flowered after three years. A massive root system was not evident in the natural populations of var. *achilleoides* that I looked at in southwestern Oregon (Appendix 1). Most were ± taprooted. Massive root systems, however, developed in greenhouse and garden plants of var. *achilleoides* populations *348* and *349*.

A massive root system was present to some degree in nature and in cultivation in the populations I treated as intermediate between vars. *achilleoides* and *leucophyllum*. That root system was best developed in Population *334* (hexaploid) and *335* and *339* (tetraploid), less well developed in *338* (diploid), except in cultivation.

Cytogeography

The diploid and polyploid populations of vars. *leucophyllum* and *achilleoides* and the *achilleoides-leucophyllum* populations identified in this study

TABLE 3. MAJOR DIFFERENCES BETWEEN MODAL POPULATIONS OF VAR. *ACHILLEOIDES* PHASE 1 AND VAR. *LEUCOPHYLLUM*.

Character	Var. *achilleoides*	Var. *leucophyllum*
Root system	taprooted, slight	fibrous, massive
Root buds	absent	abundant
Individuals	single	often clumped
Life span	annual?, biennial, short-lived perennial, often flowering the first year	longer-lived perennial, often not flowering the first year
Leaves	±crispate, gray-woolly beneath	±plane, white-woolly beneath

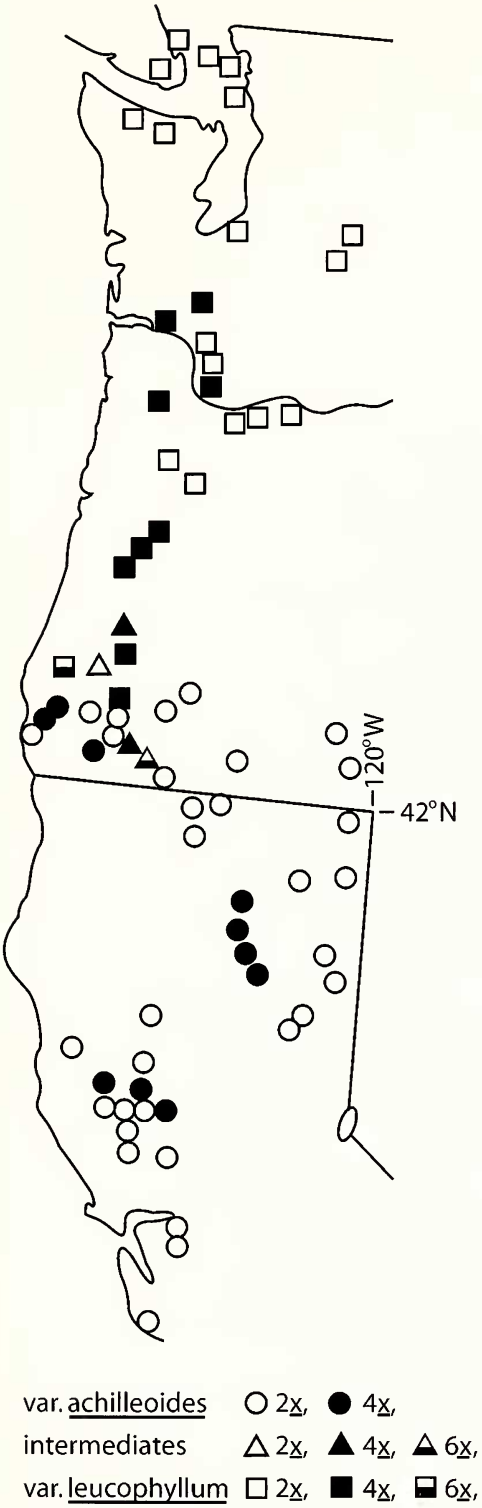
120°W
42°N
var. achilleoides ○ 2x, ● 4x,
intermediates △ 2x, ▲ 4x, ▲ 6x,
var. leucophyllum □ 2x, ■ 4x, ⬓ 6x,

FIG. 1. Distribution of diploid, tetraploid, and hexaploid populations of *Eriophyllum lanatum* var. *achilleoides*, var. *leucophyllum*, and var. *achilleoides*-var. *leucophyllum* intermediates in British Columbia, Washington, Oregon, and northern California. Not all the California populations are shown; see Fig. 1 and 2 of Mooring (2001) for more detail.

and a previous one (Mooring 2001) were not randomly distributed.

In respect to var. *leucophyllum*, the 12 northernmost cytodemes were diploid, extending from British Columbia to Pierce, Kittitas, and Yakima counties in Washington (Fig. 1, Appendices 1, 2). Southwestern Washington and northwestern Oregon had four tetraploids and five diploids. Most were close to the Columbia River. The easternmost diploid population (*342*) showed some features of var. *lanatum*, with which var. *leucophyllum* intergrades along the Columbia River. Two other var. *leucophyllum* diploid populations were farther south, in Marion County, Oregon. South of them were var. *leucophyllum* tetraploid populations in Lane, Linn, and Douglas counties and a morphologically aberrant hexaploid in Coos County.

Variety *achilleoides* cytodemes in Oregon and northernmost California were diploid except for three tetraploids in southwestern Oregon (Fig. 1, Appendices 1, 2). Differentiating between vars. *achilleoides* and *leucophyllum* becomes increasing difficult from approximately Roseburg to Ashland, Oregon. In this region, a mixed pattern of diploid and polyploid populations of both varieties and their intermediates occurs. Here, in Douglas, Josephine, and the western portion of Jackson counties, six populations were polyploid (*18*, *337*, *335*, *339*, *334*, *62*) and five were diploid (*92*, *173*, *338*, *336*, *350*). Populations *334*, *335*, *336*, *337*, *338*, *339*, and *350* were grown in greenhouse or garden culture, or both. I regarded *334*, *335*, *338*, and *339* as var. *achilleoides*-var. *leucophyllum* intermediates, *18* and *337* as var. *leucophyllum*, and *92*, *173*, *336*, *62*, and *350* as var. *achilleoides* (Appendices 1, 2; Fig. 1).

The morphological intermediacy of the polyploids and their lack of multivalents suggest allopolyploidy. I recorded counts of 17 II in Population *335*, 17–18 II in Population *337*, 23–24 II in Population *334*, and 23–25 II in Population *346*. The presence of two extra chromosomes in Population *335* may be an instance of aneuploid numbers generated by neopolyploids (see Ramsey and Schemske 2002, pp. 601–607). Alternatively, the chromosomes in excess of the basic complement may be supernumeraries, as in the diploid populations *338* and *355* (Appendix 1). The variation in number of chromosomes in Populations *337*, *334*, and *346* may reflect errors in counting chromosomes in "difficult" preparations. Alternatively, it may be a matter of aneuploid numbers or supernumerary chromosomes as noted above for Population *335*.

Supernumerary Chromosomes

Supernumerary chromosomes are known in six of the ten varieties of *Eriophyllum lanatum*.

Their frequency in individuals and populations of var. *achilleoides* was 8/85 (9%) and 8/61 (13%), respectively, in var. *leucophyllum* 6/37 (16%) and 6/29 (21%) (Mooring 2007). Artificial hybridizations have shown that the pollen and the seed parent can transmit or increase the number of supernumerary chromosomes in vars. *achilleoides* and *leucophyllum* (Mooring 2007). Three of the plants analyzed in Population *338*, intermediate between vars. *achilleoides* and *leucophyllum*, had one supernumerary chromosome; one plant had two. In artificial hybridizations these chromosomes were transmitted or increased in number (Table 1, Crosses 10 and 11).

Eriophyllum lanatum populations with supernumerary chromosomes were not randomly distributed. Their frequency was higher in regions where the varieties are sympatric or nearly so (Mooring 2007). Mooring (2007) hypothesized that these supernumerary chromosomes could originate by hybridization between chromosomally differentiated populations, especially in regions where the varieties are sympatric or nearly so. Do these supernumerary chromosomes remain in populations because they are transmitted or increased in number by meiotic mechanisms? Neutral or even deleterious gene combinations could be maintained in this way. Alternatively, I speculate, that some supernumerary chromosomes could persist in *E. lanatum* populations because they are the chromosomal equivalents of the transposable genetic elements found in maize and other species. Reserves of genetic variability are potentially adaptive, especially in hybrid zones with a variety of environments, including ecologically marginal ones, e. g., southwestern Oregon.

Barriers to Interbreeding

Components of interbreeding. In an elegant essay, Ornduff (1969) epitomized interbreeding as a succession of three related events: "relative crossability" (pollination, fertilization, and maturation of the seed), "progeny establishment" (germination, growth, and maturation of the offspring), and "fertility of the progeny." Information obtained from greenhouse and garden studies cannot be uncritically applied to interbreeding in natural populations. However, Ornduff's (1969) "relative crossability" and "progeny establishment" have a higher chance of success under greenhouse and garden conditions than under field conditions. Therefore, it seems likely that barriers to interbreeding are higher under natural conditions than under artificial ones.

Criteria for assessing barriers to interbreeding. To assess differences in barriers to interbreeding, I relied mainly on percentage pollen fertility in the progeny of the crosses and percentage change in pollen fertility between the means for the progeny and the means for their parents. Differences in percentage germination could be an important indicator of another isolating mechanism, but in the present study percentage germination could be misleading for two reasons. First, dark and thick fruits may not have a viable embryo or the embryo may not be able to penetrate the seed and fruit coats (probing the fruits to identify those with embryos improves the validity of the percentage germination criterion). Second, germination was staggered. Within a lot, most seedlings came up within 6–14 d; others required up to 30. The conditions of my study required seedlings be transplanted to pots within about three weeks after the first seedlings of that lot appeared. Other factors influencing interbreeding are meiotic aberrations and F1 vigor. In this study, conspicuous differences among progenies in meiotic pairing and F1 vigor were usually not apparent.

Caveats. 1) Assessing pollen fertility by staining techniques has limitations (see Stace 1980). Also, in this study some pollen grains in some hybrid combinations showed a continuum of intermediate conditions of plumpness and staining. I evaluated some intermediate pollen grains as fertile. Therefore, pollen fertility in some hybrid combinations may be slightly lower than my estimates. 2) As to differences in pollen fertility between parents and progeny, low pollen fertility in one parent lowers the average fertility, thus decreasing the validity of this criterion. For example, in Crosses 4 and 11 the mean pollen fertility of the progeny was higher than that of the mean of the parents. But, the pollen fertility of the parents in each cross differed substantially, 60% and 98%, and 61% and 85%, respectively. 3) Crosses other than 6 and 7 showed no evidence of chromosomal structural changes at diakinesis or first metaphase. Jackson (1984) has noted that study of pachytene stages might reveal these changes.

Barriers to interbreeding within var. achilleoides. No significant differences in mean pollen fertilities or other barriers to interbreeding were observed in var. *achilleoides* × var. *achilleoides* progenies despite the distance and environmental and other differences between the Oregon and California populations (Tables 1, 2, Crosses 1–4).

Barriers to interbreeding within var. leucophyllum. Only two interpopulation crosses were possible within var. *leucophyllum*. Both involved Oregon populations, Rowena × Silver Falls, and Rowena × Shepperd's Falls (Tables 1, 2, Crosses 5 and 6). The mean pollen fertilities of the progenies, 95% ± 5% and 81% ± 12%, respectively, differed significantly, P ≤ 0.01. Interestingly, mean percentage germination in Rowena × Silver Falls was only 8%, contrasting with its 95% pollen fertility. Some cells of a Rowena × Shepperd's Falls plant formed 7 II + 2 I, suggesting the possibility of chromosomal restructuring (Table 1).

The mean percentage pollen fertility of 95% seemed astonishingly high in the progeny of the Rowena × Silver Falls reciprocal cross. Eight of the ten plants had pollen fertilities of 95%–99%, a percentage that would be unusual even in intrapopulation crosses. The available evidence suggests that the high pollen fertility in this progeny did not result from frequent self pollination induced by human rather than natural pollinations. The mean pollen fertility of progenies of the reciprocal crosses were 96% and 94% (Table 1, Crosses 5a and 5b), and the lowest pollen fertility in any of the progenies was 84%. It is unlikely that high percentages of selfing would occur in both seed parents. Evidence supporting frequent selfing in Populations *341* and *342* is scanty. Plants of Population *341* (Silver Falls) were seed parents in a total of three other interpopulation crosses; mean pollen fertilities in the progenies were 65%–78%. Plants of Population *342* (Rowena) were seed parents in a total of six other interpopulation crosses; mean pollen fertilities in the progenies were 49%–88%. Of the total of 39 plants in both sets of progenies, only 5 had pollen fertilities of 90%–94%. Rather than frequent self pollination, the 95% mean pollen fertility in the Silver Falls × Rowena progenies most probably resulted from recombination. Recombinations yielding higher than average fertility would be advantageous under natural conditions, providing, of course, that such genotypes would be otherwise fit. See Arnold (1997, Chapter 6) for discussions of natural hybridizations leading to new evolutionary lineages.

Barriers to interbreeding between var. achilleoides and var. leucophyllum. The presence of some 7 II + 2 I configurations and a bridge-fragment in the Lower Lake × Bonneville progeny suggests the possibility of an inversion and perhaps other chromosome rearrangements. Percentage germination, however, was relatively high. The mean pollen fertilities of the two var. *achilleoides* × var. *leucophyllum* progenies did not differ significantly. The mean pollen fertility of the Lower Lake × Bonneville progeny, however, differed significantly (P ≤ 0.05) from the var. *achilleoides* × var. *achilleoides* progeny, Prospect × Los Gatos (Table 2, Crosses 3 and 7).

Barriers to interbreeding between a var. achilleoides-var. leucophyllum intermediate and var. achilleoides. Population *338*, Dillard, Oregon, is a var. *achilleoides*-var. *leucophyllum* intermediate. Plant *S338-4*, with one supernumerary chromosome, was used in the Dillard × Calistoga, California, and the Dillard × Galice, Oregon crosses. The progeny included plants with one or two supernumerary chromosomes, indicating that these chromosomes can be transmitted or increased in number (Mooring 2007). Mean pollen fertilities of the progenies were similar, 69% ± 11% and 64% ± 20%, respectively. These means were 22% and 19% lower, respectively, than the averages of their parents (Table 1, Crosses 9 and 10). Mean pollen fertility in the Dillard × Calistoga progeny differed significantly (P ≤ 0.05) from the following var. *achilleoides* × var. *achilleoides* progenies: Calistoga × Calistoga, Litchfield × Prospect, and Prospect × Los Gatos) (Table 2, Crosses 1, 3, 4, and 9). The Calistoga and Los Gatos populations are Phase 1 of var. *achilleoides*; the Litchfield and Prospect populations are Phase 2. In Cross 10, Dillard × Galice, mean pollen fertility of the progeny did not differ significantly from the var. *achilleoides* × var. *achilleoides* progenies noted above (Table 2). Percentage germination, however, was 3%, and only 4 of the 15 progeny flowered (Table 1).

Barriers to interbreeding between a var. achilleoides-var. leucophyllum intermediate and var. leucophyllum. Population *338*, Dillard, Oregon, is a var. *achilleoides*-var. *leucophyllum* intermediate. Plant *S338-6*, with two supernumerary chromosomes, was crossed to a var. *leucophyllum* plant from a Rowena, Oregon population. One or two supernumeraries were transmitted to five of the six progeny (Mooring 2007). Mean pollen fertility of the progeny (81% ± 13%) was significantly higher (P ≤ 0.01) than that of the Population *338* × var. *achilleoides* progeny (69% ± 11%), suggesting that barriers to interbreeding are less well developed between Population *338* and var. *leucophyllum* than between Population *338* and var. *achilleiodes* (Tables 1, 2, Crosses 9, 10, and 11).

Comparisons of pollen fertilities. Mean pollen fertilities in the progenies of diploid crosses involving var. *achilleoides*, var. *leucophyllum*, and Population *338*, intermediate between the two varieties, were 64%–81%, averaging 78% (Table 1), much higher than mean pollen fertilities reported for other intervarietal crosses in the *E. lanatum* complex (Mooring 2001). In the 2001 study, ranges for low, medium, and high mean pollen fertilities were set at 22%–40%, 42%–58%, and 60%–76%, respectively (Mooring 2001, Table 7). Barriers to interbreeding do not seem to be well developed among var. *achilleoides*, var. *leucophyllum*, and Population *338*, if pollen fertility is used as a criterion for assessing such barriers. However, the possibility that the supernumerary chromosomes in Population *338* promoted higher pollen fertility has to be considered.

CONCLUSION

The geographic distribution of diploid and polyploid populations in *E. lanatum* vars. *achilleoides* and *leucophyllum*, and intermediates between these two taxa in southwestern Oregon portrays a familiar pattern in the *E. lanatum* species complex: polyploid populations predominate in regions where infraspecific taxa meet. The relatively high pollen fertility in the progenies of artificial hybrids in this study suggests that barriers to interbreeding between vars. *achilleoides* and *leucophyllum* are relatively low in southwestern Oregon. The capacity to produce fertile hybrids from crosses between taxa accounts for some of the difficulty in deciding on taxonomic designations for intermediate populations. Also, low barriers to interbreeding increase the probability of producing genotypes leading to new evolutionary lineages, the "evolutionary novelties" of Arnold (1997, Chapter 6). The geological antiquity, relatively benign climate, and the topographic and edaphic diversity of southwestern Oregon (Hunt 1974) increase the chances of new and successful evolutionary lineages. The populations characterized as "*Eriophyllum ternatum*" (see Cronquist 1955, p. 196) may be examples of such lineages.

Polyploid populations in Oregon largely bridge the gap between the diploid var. *leucophyllum* populations of British Columbia and Washington, and the diploid var. *achilleoides* populations of northern California and southwestern Oregon. If the Oregon polyploid populations identify a history of natural hybridizations, then the hybrid zone may extend from the Columbia River to the Siskiyou Mountains of southwestern Oregon and northwestern California. Some features of what I have termed "Phase 2" of var. *achilleoides* may reflect the introduction of genes from var. *leucophyllum*.

Molecular level studies might define the extent of the hybrid zone, indicate whether vars. *grandiflorum* and *lancelolatum* are involved in the taxonomic complexity in the Siskiyou Mountains, and find if clines are present.

ACKNOWLEDGMENTS

The late Kathryn Halloran Mooring helped with field work, expert proof reading, and other assistance. Craig Stephens and Michelle Marvier provided laboratory facilities after retirement. An internal grant supported the project. I thank Robert Numan for his guidance with statistical procedures, Kenton Chambers for his knowledge of Oregon botany, Katherine Preston for suggestions on organizing the manuscript, John Strother for editorial expertise in pre-submission reviews, and Kenton Chambers and an anonymous reviewer for their post-submission comments.

LITERATURE CITED

ANDERSON, L. 1966. Cytotaxonomic studies in *Chrysothamnus* Astereae, Compositae). American Journal of Botany 53:204–212.

ARNOLD, M. 1997. Natural hybridization and evolution. Oxford University Press, New York, NY.

CONSTANCE, L. 1937. A systematic study of the genus *Eriophyllum* Lag. University of California Publications in Botany 18:69–136.

CRONQUIST, A. 1955. Vascular plants of the Pacific Northwest, Part 5: Compositae. University of Washington Press, Seattle, WA.

HUNT, C. 1974. Natural regions of the United States and Canada. W. H. Freeman, San Francisco, CA.

JACKSON, R. 1984. Chromosome pairing in species hybrids. Pp. 67–86 *in* W. F. Grant (ed.), Plant systematics. Academic Press, New York, NY.

MOORNING, J. 2001. Barriers to interbreeding in the *Eriophyllum lanatum* (Asteraceae, Helenieae) species complex. American Journal of Botany 88:285–312.

———. 2007. Supernumerary chromosomes in *Eriophyllum lanatum* and *E. confertiflorum* var. *confertiflorum* (Asteraceae). Madroño 54:30–41.

ORNDUFF, R. 1969. The systematics of populations in plants. Pp. 104–128 *in* Systematic Biology, Proceedings of an International Conference. National Academy of Sciences, Washington, DC.

RAMSEY, J. AND D. SCHEMSKE. 2002. Neopolyploidy in flowering plants. Annual Review of Ecology and Systematics 33:589–639.

STACE, C. Plant taxonomy and biosystematics. Edward Arnold, London, U.K.

APPENDIX 1

UNPUBLISHED 2*N* CHROMOSOME COUNTS IN THE *ERIOPHYLLUM LANATUM* COMPLEX

The county name follows the state name. The first number is that of the population, the second is my collection number. Voucher specimens have been deposited in SACL. Counts are for one plant, unless a number in parentheses shows more. Locations are approximate. Populations used in artificial hybridizations (Tables 1, 2) are in bold print.

***E. lanatum* (Pursh) Forbes var. *achilleoides* (DC.) Jepson**

OREGON: JOSEPHINE, *336*, *3999*, 8 II, Granite Hill Road, Grants Pass; ***350*, *4017*, 8 II (2), near Galice.** CURRY, ***348*, *4015*, 16 II, hwy 33 between Gold Beach and Agness**; *349*, *4016*, 16 II (2), junction of hwys 33 and 23 near Agness.

***E. lanatum* (Pursh) Forbes var. *leucophyllum* (DC.) W. R. Carter**

OREGON: MARION, ***341*, *4004*, 8 II, near Silver Falls State Park**. MULTNOMAH, ***355*, *4030*, 8 II, 8 II + 2 I, near Shepperd's Dell State Park**. WASCO, ***342*, *4005*, 8 II (3), near Rowena along old hwy 30**. DOUGLAS, *337*, *4000*, 16 II, 17–18 II, near Azalea. LANE, *340*, *4003*, 16 II, between Elmira and Monroe.

COOS, *346*, *4013*, 23–25 II, near Bridge.

WASHINGTON: WAHKIAKUM, ***344*, *4011*, 16 II, along Columbia River near Cathlamet**.

Var. *achilleoides*-var. *leucophyllum* intermediates.

OREGON: DOUGLAS, ***338*, *4001*, 8 II + 1 I (3), 8 II + 2 I or 9 II, near Dillard**; *339*, *4002*, 16 II, near Yoncalla.

JACKSON, ***335*****,** ***3998*****, 17 II, near Central Point**; *334*, *3997*, 23–24 II, near Emigrant Lake.
E. lanatum var. *grandiflorum* (A. Gray) Jepson
OREGON, JOSEPHINE, *351*, *4018*, 8 II, near O'Brien on road to Happy Camp.
E. *lanceolatum* (Howell) Jepson
OREGON, JOSEPHINE, *352*, *4019*, 8 II, ca. 15 mi N of Happy Camp.
CALIFORNIA, SISKIYOU, *353*, *4020*, 8 II, 9 mi N of Happy Camp on road to O'Brien.

APPENDIX 2

PREVIOUSLY PUBLISHED 2*N* CHROMOSOME COUNTS IN THE *ERIOPHYLLUM LANATUM* COMPLEX

The name of the county follows that of state. The first number is that of the population, the second is my collection number. Voucher specimens have been deposited in SACL. Locations are approximate. Populations represented in artificial hybridizations (Tables 1 and 2) are in bold print.

E. lanatum **(Pursh) Forbes var.** ***achilleoides*** **(DC.) Jepson**
CALIFORNIA: Colusa, ***149*****,** ***2073*****, Wilbur Springs, 16 II**. Lake, ***120*****,** ***1913*****, Kelseyville, 8 II;** ***121*****,** ***1916*****, Lower Lake, 8 II**. Lassen, ***242*****,** ***3125*****, Litchfield, 8 II**. Napa, ***118*****,** ***1908*****, Calistoga, 8 II**. Santa Clara, ***38*****,** ***1375*****, Los Gatos, 8 II (5)**.
OREGON: Curry, ***347*****,** ***4014*****, Gold Beach, 8 II**. Jackson, *173*, *2158*, Mt. Ashland, 8 II; ***275*****,** ***2495*****, Prospect, 8 II**. Josephine, *92*, *1576*, Sunny Valley, 8 II; *62*, *2260*, Selma, 16 II.

E. lanatum **(Pursh) Forbes var.** ***leucophyllum*** **(DC.) W. R. Carter**
OREGON: Douglas, *18*, *1056*, Whistlers Bend State Park, 16 II, 16 II + 1 I. Multnomah, ***167*****,** ***2147*****, Bonneville, 8 II**.